

# EXHIBIT 72



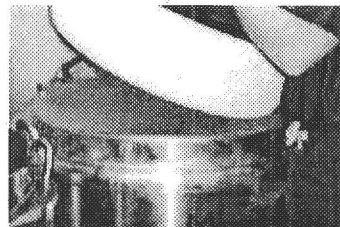
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## Sperm, Egg, and Embryo Freezing

### Freezing of human sperm, eggs and embryos

Within the laboratory at PFC we have a tissue bank where we freeze and store oocytes (eggs), sperm and embryos for our patients. The bank has a full time manager under supervision of the lab director, and is licensed and inspected by the State of California. All tissue in the bank is stored frozen in liquid nitrogen at a temperature of  $-196^{\circ}\text{C}$  in vacuum lined tanks that are computer controlled and monitored 24 hours a day with a state of the art alarm system. The embryologists are responsible for maintaining the bank and no other PFC employee has access.



### Freezing Sperm

Scientists have been freezing sperm for decades and as a result the technology for preserving and storing sperm is well defined. At PFC, we are freezing sperm samples almost every day, which are then stored frozen until they are needed. We also receive frozen sperm from sperm banks and other tissue banks that we store until the patient is ready to use. Sperm tolerate the freezing process well, although we do see some variation from patient to patient. Many patients freeze sperm for convenience (say a husband knows that he will be travelling on the day we will be performing an insemination on his wife), but for most patients there is a specific reason to freeze the sperm. We perform surgeries for some men to harvest sperm from the epididymis or testicle, and this sperm is frozen in several aliquots to help the patient avoid having to have the surgery again in the future. For others, sperm numbers are low and we try to accumulate sperm in the freezer to allow the couple to do inseminations or IVF. We also will suggest sperm freezing for any man who may be anxious about producing a sample on the day of his partner's insemination or egg retrieval.

When sperm is thawed from the bank it is normal for some of the sperm to die. Not all cells survive freezing and thawing, but since most sperm samples have many millions of live sperm, losing a small fraction of them has little consequence. However, it is important to be aware of the expected drop in the number of live sperm after freezing so that enough sperm can be frozen up front for the purpose of having one or more pregnancies. If you reference the visual representation of the process of freezing sperm above, you will see that the sperm are stored in carefully labeled vials (1) and placed in a cooling tank (2). The vials are then placed in a tray (3), slid into a labeled storage drawer (4) and lowered into a nitrogen tank (5).

### Freezing and storing human embryos

Our experience with freezing embryos is not quite as substantial as with freezing sperm. Nevertheless, since the first human embryos were successfully frozen back in 1984, hundreds of thousands of babies have been born from embryos that spent some time in the freezer. Embryo freezing is a very routine part of the IVF process and approximately 60% of patients end up with some embryos in storage.

Freezing of excess good quality embryos after IVF allows for the transfer of fewer embryos in the stimulated IVF cycle and, therefore, ensures fewer high-order (triplets or more) multiple births. This process provides patients with a "back-up" should the initial fresh embryo transfer not result in a pregnancy, at a much lower cost than starting IVF all over again and often with minimal medications. Frozen embryo transfers have allowed many of our patients to achieve more than one pregnancy from a single cycle of ovarian stimulation.

Embryos can be frozen at any stage of development during the IVF process. Eggs that are fertilized can be frozen as early as 1 day after an egg retrieval procedure, but it is more common to allow embryos to develop for a number of days before freezing them. This allows us to observe how well the embryos are developing so that we only end up with embryos in the freezer that we think have a good probability of establishing a pregnancy.

Embryos tolerate freezing very well and we expect over 90% of embryos to survive the process. Pregnancy rates with frozen/thawed embryos are as good as pregnancy rates for embryos that were transferred fresh without ever being frozen.

#### Common Questions about freezing:

[How are embryos frozen? \(#search%20embryos%20frozen\)](#)

[What are the specific steps in embryo freezing? \(#Specific%20Steps\)](#)

[How are frozen embryos stored and monitored? \(#Stored%20and%20monitored\)](#)

[How long can embryos be stored? \(#How%20long%20stored\)](#)

[How are embryos thawed? \(#Thawed\)](#)

[Can freezing damage my embryos? \(#Freezing%20damages\)](#)

[Am I more likely to have a child with a genetic or congenital abnormality because I'm pregnant after a frozen embryo transfer? \(#Can%20be%20infectious\)](#)

[What are the costs for keeping embryos in frozen storage? \(#Costs\)](#)

[Would the storage tanks survive a major earthquake or other disaster? \(#Disaster\)](#)

[What are my options for using the embryos? \(#What%20options\)](#)

[How are embryos destroyed when patients request disposition? \(#Destroyed\)](#)

[Can I donate my embryos to another infertile couple? \(#Donate\)](#)

[Shipping embryos to or from PFC? \(#Shipping\)](#)

#### How are embryos frozen?

The main concern during the freezing of any cell is the removal of water without actually killing the cell. Since water expands in volume as it freezes, ice formation inside a cell would cause the cell to rupture and die. Therefore, cell water is traditionally replaced with a cryoprotectant (antifreeze) prior to cooling of the cell. This is achieved by sequentially incubating the cell in increasing concentrations of cryoprotectant. The cryoprotectant draws water out of the cell and itself enters the cell, all by osmosis. Once most of the water has been removed, the cell is cooled at the very slow rate of  $-0.3^{\circ}\text{C}/\text{minute}$  until it has been cooled to below  $-30^{\circ}\text{C}$  and is therefore fully frozen. Thereafter, storage of frozen cells is in liquid nitrogen ( $-196^{\circ}\text{C}$ ), which is a simple and practical storage medium.

The newer method, vitrification, still requires the use of cryoprotectants and the cell is also ultimately stored in liquid nitrogen, but the journey from the incubator (at  $37^{\circ}\text{C}$ ) to the nitrogen ( $-196^{\circ}\text{C}$ ) is much faster. The word "vitrum" in Medieval Latin means "glass" and the process turns the cell contents to a glass like substance instead of ice. Since little or no ice forms, the risk of rupturing the cell is eliminated. For glass to form instead of ice, the rate of cooling must be thousands of degrees per minute instead of the  $0.3$  degrees/minute that we use in slow freezing. Therefore, the process is sometimes referred to as ultra-rapid freezing, although the word "freezing" is really inappropriate here since the cell is not really frozen (i.e. no ice is created).

One of the big stumbling blocks during egg freezing was the sheer size of the cell (the egg is the largest human cell by some margin) and, therefore, it has a high water content. Just getting the cell to survive, (an egg is only one cell), was an incredibly difficult obstacle to overcome. Studies where 50-60% of the eggs survived were considered groundbreaking, and in the 1990's and early 2000's there are few studies that have done better. The IVF community largely ignored vitrification as an effective technique because it was technically more challenging. But, with success rates using traditional slow freezing failing to improve, vitrification was given serious consideration as an alternative. In the few years since its introduction, vitrification has shown promising and excellent results in clinical studies (see Oktay et al., Fertility and Sterility, 2006, Vol 86(1), pages 70-80 a comparative review of slow freezing and vitrification results with human oocytes).

Making the transition from slow freezing to vitrification has been a challenge for the IVF community. As already stated, it is a technically challenging procedure, and training of embryologists in the technique has been slow. With slow freezing, embryos are placed in relatively weak solutions of cryoprotectant for as long as 5 minutes at a time. Then, they are usually moved on through slightly stronger solutions before being placed in large straws or vials, which are then loaded into a computer-controlled freezer for the long slow journey to  $-30^{\circ}\text{C}$ . The embryologist can spend about 30 minutes with a set of embryos from the time that



they come out of the incubator until they go into the controlled rate freezer. After 2 or more hours, the embryos can be placed in liquid nitrogen and the process is complete.

During a vitrification procedure, where typically only one egg or embryo can be worked on at a time, the transition from incubator to nitrogen takes only a few minutes. The embryo is stepped through solutions containing incrementally higher concentrations of cryoprotectants where it shrivels and swirls in the extremely viscous medium. In the final stage, which is measured in seconds, the egg or embryo is placed in a highly concentrated cryoprotectant solution and then quickly loaded up into a tiny straw that is barely larger than the egg or embryo itself. The straw is then sealed at both ends and plunged immediately into liquid nitrogen. The straw is so fine that it freezes in an instant, an important part of the vitrification process. The loading of the straw occurs at room temperature (25° C in the IVF lab) and it is cooled to -196° C in two or three seconds, giving a cooling rate of 4000-6000° C/min. The faster the straw can be cooled, the more successful the procedure. Performing this final step too slowly or too quickly can be the difference between success and failure and therefore requires extensive training.

At Pacific Fertility Center, we have been working on vitrification since 2007. Our initial interest was in egg freezing, with the intent to extend the technique to embryos, particularly at the blastocyst stage in which slow freezing has not always worked optimally. Slow freezing has served us well over the years for embryos frozen 1, 2 or 3 days after egg retrieval. However, blastocysts, 5 to 6 day-old embryos, have not fared as well with slow freezing. Since the majority of IVF patients now have blastocyst stage embryo transfers, we now look at vitrification as the best method of preservation for these precious embryos.

A blastocyst is an embryo that has developed to the stage where it is ready to implant in the uterus. Instead of having a small number of loosely associated cells characteristic of earlier embryonic stages, it has 2 defined cell populations and a fluid filled cavity (or cyst). The cells that surround the cavity will form the placenta, and the cells within the cavity will develop into the embryo proper (or fetus), and some of the extra-embryonic membranes, such as the yolk sac. It is these interior cells that cause trouble during freezing since they are on the inside and difficult to expose to cryoprotectant. Slow freezing relies on cryoprotectant traveling through the outer placental cells, then the cavity, and finally into the fetal cells, while water travels in the opposite direction. Fully dehydrating these fetal cells has always been a challenge and an embryo where these cells do not survive freezing and thawing will not result in a viable pregnancy.

After investing heavily in vitrification training and implementing a successful egg vitrification program, PFC began working on blastocyst vitrification in January of 2007. By March we had a program established and were delighted by how easily blastocysts seemed to tolerate the procedure. Often, blastocysts looked no different after vitrification when compared to how they looked before the procedure. These results stood in stark contrast to the results of slow freezing where blastocysts sometimes looked shriveled and deflated upon exiting the freezer, with some or all cells dead or dying. By July 2007 we had switched completely to vitrification and are currently enjoying the success that it brings to our patients and our practice.

Our initial vitrification team consisted of only 3 embryologists because of the technical challenges involved. However, as we realized that vitrification was going to be the way all embryos would be preserved going forward, the rest of the embryology team were trained over a 1 year period. The IVF lab is open every day (365 days a year), so having everybody proficient with vitrification is very important. Furthermore, we have developed techniques to use a laser to artificially collapse the blastocyst cavity prior to freezing, allowing the cryoprotectant to enter the cavity and relieving pressure on the internal cells. The blastocysts then re-expand upon thawing and do exceptionally well with implantation success.

Vitrification is now the method of choice for freezing eggs and embryos at all stages of development in the lab at PFC. The procedure works very well with embryo survival rates consistently above 95% year after year. Embryos tolerate the procedure very well and pregnancy rates with vitrified embryos are as good as or better than with fresh embryos. And knowing that vitrified embryos do so well, many patients are choosing to transfer just one embryo in their fresh cycle to minimize their risks of having a multiple pregnancy.

#### **What are the specific steps in embryo freezing?**

**Step 1.** Embryos are removed from the 37°C incubator and placed in a weak solution of CPA's at room temperature. Embryos spend 8 minutes in this solution, which is enough time for the water and CPA's to equilibrate. When first in the CPA solution, embryos initially shrink (as water leaves the embryo) and then re-expand as the CPA's enter the cells.

**Step 2.** Embryos are placed in a stronger CPA solution and allowed to equilibrate for just 1 minute.

**Step 3.** Each embryo is loaded up into a fine straw which has been pre-labeled with identifying and tracking information. The label has information on the individual(s) that created the embryo (full names and date of

birth), tracking information for any sperm and/or oocyte donor used, the date of the freezing procedure and the status of the embryo at the time of freezing.

**Step 4.** The straw is sealed at both ends and plunged into liquid nitrogen at a temperature of  $-196^{\circ}\text{C}$ . It will remain at this deep sub-zero temperature until the individual(s) that created the embryo request for it to be warmed.

#### How are frozen embryos stored and monitored?

The air that we breathe contains a gas called nitrogen. This gas makes up about 78% of the air around us. If nitrogen gas is cooled, it becomes liquid at  $-196^{\circ}\text{C}$ . This liquid is very stable and easy to work with. In the laboratory we have large tanks filled with liquid nitrogen in which we store frozen embryos. Each tank is in effect like a large thermos flask since it is vacuumlined. All patients have designated storage spaces within a tank, where their embryos are kept. The straws that contain the embryos are color-coded and labeled with precise and unique identifying information as described above.

The tanks that contain frozen embryos are monitored 24 hours a day, 7 days a week, 365 days a year. Each tank gets a physical inspection twice a day, looking for problems or signs of wear. The quantity of nitrogen in the tank is assessed as a means of monitoring for a possible slow leak or an impending tank failure. The nitrogen in the tank is topped up once or twice a week, since it continuously evaporates at a slow rate (if a tank was not filled regularly, the nitrogen would evaporate entirely in about 6 weeks).

Electronic tank monitoring uses 3 different sensors to ensure that tanks perform to specifications. A probe that is attached to the tank lid actually sits in the nitrogen with the embryos. The probe will detect a rise in temperature within the tank, or a drop in the level of liquid in the tank. The laboratory also has an oxygen alarm that will detect when nitrogen is evaporating at a high rate and displacing oxygen from the air. All 3 of these sensors are connected to a telephone alarm system that will alert staff to an alarm condition outside of normal working hours.

The telephone alert system is a complicated monitoring device. It requires that 8 people be contactable at any given time, and calls and recalls each person in turn until somebody enters the laboratory and cancels the alarm. The alarm cannot be canceled remotely, and our protocol requires that an embryologist be in the laboratory no more than 30 minutes (day or night) after the alarm is set off. The alarm system is tested every day and continues to run on battery power in the event of a power failure. The alarm system can also be checked remotely. The status of each individual tank can be ascertained by telephone at any time.

#### How long can embryos be stored?

No one knows what the maximum storage period might be. Procedures for human embryo freezing were developed in 1984 and only went into widespread use in the late 1980s. This means that the longest time a human embryo has been stored is 25-30 years and, typically, patients that have left embryos in storage for this long are not coming back for them. Some patients have come back after 10-12 years and the embryos have been thawed successfully and created healthy babies. Beyond this time frame, we don't know how long an embryo will remain viable, but it is possible that, kept in liquid nitrogen, an embryo may be viable indefinitely.

#### How are embryos thawed?

The process of embryo freezing has already been explained. Thawing the embryos is simply a reversal of the freezing procedure.

Thawing includes our SurTransfer<sup>TM</sup> protocol. When an embryologist removes embryos from the freezer, a second embryologist is required to witness the act, and verify the identity of the embryos before they can be thawed. Under no circumstances can a lone embryologist remove embryos from the freezer without another embryologist to confirm correct identification.

The embryos coming out of the freezer (at  $-196^{\circ}\text{C}$ ) are warmed to room temperature in 3 seconds. This rapid thaw method minimizes damage to the embryo from ice that could form during warming. The embryologist has to remove the antifreeze from the embryo and replace the water that was removed at the time of freezing. This is done by incubating the embryo in decreasing concentrations of the antifreeze, and increasing concentrations of water. Over a period of 20 minutes, the embryo is stepped through different solutions, until finally the antifreeze is gone and all the water has been replaced.

The thawing procedure is performed at room temperature, and once complete, the embryo is warmed up to



body temperature (37°C). It can be ready for transfer in as little as 40 minutes after leaving the freezer.

#### **Can freezing damage my embryos?**

While every care is taken to protect the embryos during the process, some embryos may have one or more dead cells after they have been thawed. At PFC, we currently have a 98% survival rate after warming vitrified embryos. We consider a warmed (thawed) embryo as having a normal chance of implanting after transfer.

#### **Am I more likely to have a child with a genetic or congenital abnormality because I'm pregnant after a frozen embryo transfer?**

Even after 20 years, there are few studies in the scientific and medical literature concerning outcomes after embryo cryopreservation. However, the few studies that have been published are thus far very reassuring. Children born from frozen embryos do not seem different from children born from embryos that had not been frozen. Even if an embryo loses some cells during thawing this does not cause any abnormalities. Freezing does not cause or introduce genetic abnormalities.

#### **What are the costs for keeping embryos in frozen storage?**

The costs to Pacific Fertility Center are considerable. Most of the cost is accounted for by the manpower required to maintain the tanks and manage the inventory of stored embryos. Embryologists receive special training in handling liquid nitrogen and maintaining the frozen embryo bank. Although nitrogen liquid is relatively stable, it can cause severe frostbite, rapid suffocation and death if mishandled. We even have a special permit from the city just to have the liquid in the building and the emergency services have to be kept informed of our activities.

We buy several hundred liters of nitrogen each week to keep the tanks filled and to use for freezing of new embryos. Tanks are serviced, replaced and maintained according to a strict schedule and alarm systems are also maintained to a very high standard. Ask any embryologist, and they'll tell you that the real cost is in having to race to the lab at 3:00 am on a Saturday morning only to find a false alarm.

#### **Would the storage tanks survive a major earthquake or other disaster?**

Probably not. While the tanks are secure and robust, they could be crushed or severely damaged by falling masonry. Any catastrophe that would collapse the building would almost certainly destroy the tanks.

The storage tanks require no power and would not be impacted by a power failure or blackout. They are made of metal and would probably survive a small or moderate fire. If the tanks were not physically damaged or knocked over in a disaster, they should survive intact. Even if no one was able to physically check the tanks, or if we were unable to obtain liquid nitrogen, the tanks should still hold their temperature for several weeks.

#### **What are my options for using the embryos?**

We hope that most couples will be able to use the embryos to have a healthy baby. Patients undergoing frozen embryo transfer make up about one fourth of the patients visiting our office. Some are thawing embryos after failing to become pregnant during their IVF cycle, and some are using the embryos a year or more after a successful IVF cycle, to have a second or third child.

You may be surprised to learn that there are a significant number of people who do not want to use their frozen embryos to become pregnant. These are typically people that have completed their families and are not interested in having any more children. Having embryos remaining creates a very difficult situation for these families. The embryos can be discarded as medical waste, but the decision to destroy the embryos is not made easily. Couples with children resulting from IVF treatment often view the frozen embryos as potential children and siblings for their existing children. Coming to terms with destroying the embryos can be very difficult, and many couples avoid taking this decision by simply leaving the embryos frozen indefinitely. In the UK, the government has taken action against these couples by ordering the destruction of all embryos in frozen storage for more than 5 years. No such laws or actions have, as yet, been undertaken in the US. Nonetheless, it is very important for all couples and individuals to think about what they might eventually do with excess embryos in the future should they find themselves in this situation. Some may want to freeze some eggs rather than fertilizing all eggs up front. Although a potentially costlier solution, it could help to avoid having to make an impossible choice at some future point.

Frozen embryos can also be donated for research studies. Embryos donated for research will be thawed

and used in a scientific study, and discarded after a few days. A research study might look at new ways of freezing or thawing embryos, new ways of growing embryos in the laboratory or at the genetic make up of the embryos. The studies will not benefit the patient that donates the embryos, but the research may benefit other IVF couples in the future.

#### **How are embryos destroyed when patients request disposition?**

A formal request to destroy the embryos must be received in writing from the patients. The request must be signed by both partners and notarized, or witnessed by a member of our staff. Once the laboratory has received the disposition notice, no action is taken for 30 days. This gives the couple a cooling off period and an opportunity to change their decision.

When the 30-day waiting period has passed, two Embryologists take responsibility for carrying out the patients' wishes. They fill out a form indicating that they have checked the disposition request and are in agreement that the patient wants the embryos discarded. They locate the embryos in the storage tank and double check the identity with the paperwork. The embryos are then thawed and discarded. The paperwork is complete when both embryologists sign, attesting that they performed and witnessed the destruction according to the patients' wishes. The paperwork is kept in the laboratory files and a copy filed in the medical record of the patient.

#### **Can I donate my embryos to another infertile couple?**

Yes, it is possible to donate embryos. This process is referred to as embryo donation.

There are many advantages to embryo donation, including giving another couple the chance to have a child and avoiding having to discard the embryos that took so much effort to create. Couples wanting to donate surplus embryos can donate them anonymously to other couples through PFC. Your Physician can explain the process to you.

Pacific Fertility Center does not typically get involved in open adoptions (where the donating and receiving couples know each other and/or want to stay in contact). We can help with medical tests and performing the actual thawing and transfer of the embryos, but patients may be required to get legal advice and psychological testing before an open donation can proceed. Often, the embryos will have to be moved to another part of the country, since a recipient could potentially be in any part of the country. In this situation, shipping the embryos is the responsibility of the donating couple, and we can provide information on getting embryos sent to another IVF clinic.

Couples considering open embryo donation can find information on this subject on the Internet at [Snowflakes Embryo Adoption](http://www.snowflakes.org) ([www.snowflakes.org](http://www.snowflakes.org)) or [Embryo Adoption Awareness Center](http://www.embryo-adoption-awareness.com) (<http://www.embryo-adoption-awareness.com>) and from [RESOLVE](http://www.resolve.org) ([www.resolve.org](http://www.resolve.org)), a national organization for childless couples.

#### **Shipping embryos to or from PFC?**

We do not encourage patients to move frozen embryos from one clinic to another since there is always the possibility that embryos can be lost or damaged during shipment. Among the major national/international shippers (FedEx, UPS, DHL etc), none of them will knowingly accept human embryos for shipment and insurance cannot be obtained to cover movement of embryos from one site to another.

If embryos have to be moved, they are transported inside a small tank called a dewar. A dewar is a miniature version of the large tanks in which embryos are routinely stored, but the liquid nitrogen required for cooling is held securely inside special absorbent walls in the dewar. This prevents the liquid from spilling or being lost during shipping, and reduces the safety risks for personnel handling the tank. Since a dewar is a relatively small tank, it can only maintain the required sub-zero temperature for about 7 days, before more liquid nitrogen needs to be added. This limitation is especially critical for long distance shipments where there could be delays or customs problems that could make a journey last 7 days or longer. As a result, we strongly encourage all embryo shipments to be completed in the shortest possible time, so that the embryos have the best chance to arrive still frozen at the recipient clinic. Any delay could mean loss of all the embryos.